Evaluation of the Vitek 2 ID-GNB Assay for Identification of Members of the Family *Enterobacteriaceae* and Other Nonenteric Gram-Negative Bacilli and Comparison with the Vitek GNI+ Card

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We evaluated the Vitek 2 ID-GNB identification card (bioMérieux, Inc., Durham, N.C.) for its ability to identify members of the family Enterobacteriaceae and other gram-negative bacilli that are isolated in clinical microbiology laboratories. Using 482 enteric stock cultures and 103 strains of oxidase-positive, gram-negative glucose-fermenting and nonfermenting bacilli that were maintained at -70° C and passaged three times before use, we inoculated cards according to the manufacturer's directions and processed them in a Vitek 2 instrument using version VT2-R02.03 software. All panel identifications were compared to reference identifications previously confirmed by conventional tube biochemical assays. At the end of the initial 3-h incubation period, the Vitek 2 instrument demonstrated an accuracy of 93.0% for the identification of enteric strains; 414 (85.9%) were correctly identified at probability levels ranging from excellent to good, and an additional 34 (7.1%) strains were correctly identified but at a low level of discrimination. Nineteen (3.9%) strains were unidentified, and 15 (3.1%) were misidentified. The 19 unidentified strains were scattered among 10 genera. Three of the 15 misidentified strains were lactose-positive Salmonella spp. and were identified as Escherichia coli; another was a lactose-positive, malonate-negative Salmonella enterica subsp. arizonae strain that was identified as E. coli. Of the 103 glucose-fermenting and nonfermenting nonenteric strains, 88 (85.4%) were correctly identified at probability levels ranging from excellent to good, and 10 (9.7%) were correctly identified, but at a low level of discrimination, for a total of 95.1% accuracy with this group. Two strains were unidentified and three were misidentified. The errors occurred for strains in three different genera. With the increased hands-off approach of the Vitek 2 instrument and accuracies of 93% for the identification of enteric organisms and 95.1% for the identification of nonenteric organisms with the ID-GNB card, use of this product presents an acceptable method for the identification of most gram-negative organisms commonly isolated in the clinical laboratory. A comparison of these results to those obtained by testing 454 of the same strains with the Vitek GNI+ card revealed no significant difference in the abilities of the two cards to identify these organisms accurately.

Because relatively few commercially available molecular methods for the identification of clinically significant gramnegative bacilli in the clinical laboratory exist today, the need for identification procedures that use more conventional processes remains. Some of these phenotypic identification procedures are based on colorimetric or pH-based changes and usually require 18 to 24 h to identify organisms. Some are based on changes in preformed enzymes, shortening to 2 to 4 h the time necessary to make an identification.

The newest instrument for bacterial identification and susceptibility testing is Vitek 2 from bioMérieux, Inc. (Durham, N.C.). This is a fully automated system designed to decrease the turnaround time for the identification of bacteria and determination of antimicrobial susceptibilities. The instrument

also provides a more hands-off approach than the original Vitek instrument.

The Vitek 2 ID-GNB card is a 64-well card designed for the automated identification of most clinically significant fermenting and nonfermenting gram-negative bacilli.

Because no U.S. studies have evaluated this instrument with an extensive organism library, we tested the ability of the Vitek ID-GNB card and the Vitek 2 instrument to identify strains of the family *Enterobacteriaceae* as well as glucose-fermenting and -nonfermenting gram-negative bacilli.

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MATERIALS AND METHODS

Culture collection. A total of 482 biochemically typical and atypical isolates of the family *Enterobacteriaceae* from the stock culture collection of the Centers for Disease Control and Prevention (CDC) were taken from storage in defibrinated sheep blood at -70° C and passaged three times on tryptic soy agar with 5% sheep blood (TSA II; Becton Dickinson Biosciences, Inc., Sparks, Md.) before use. In addition, 103 biochemically typical and atypical nonenteric, glucose-

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TABLE 1. Enteric strains tested with the ID-GNB card

Reference identification	No. (%) of strains identified at the following probability level ^a :								
	n	Excellent	Very good	Acceptable	Good	Low	Unidentified	Error	
Buttiauxella agrestis	2	1	1						
Cedecea davisae	6	2	2	1			1		
Cedecea lapagei	4	3		1					
Citrobacter amalonaticus	10	3	4			2		1	
Citrobacter braakii	6	2	1	1		1		1	
Citrobacter farmeri	5	3				2			
Citrobacter freundii	2	2							
Citrobacter koseri	10	4	2	1	2		1		
Citrobacter youngae	5	1	1		2	1			
Edwardsiella tarda	10	4	1		2	2		1	
Enterobacter aerogenes	10	4	3	1	1		1		
Enterobacter asburiae	10	5	2		2		1		
Enterobacter cancerogenus	10	4	2		1	1	1	1	
Enterobacter cloacae	10	2	3		1	3		1	
Enterobacter gergoviae	10	6	3		1				
Enterobacter sakazakii	10	3	2	5					
Escherichia coli	30	7	10	2	5	2	3	1	
Escherichia fergusonii	10	7	1	2					
Escherichia hermannii	10	4	1		4		1		
Escherichia vulneris	10	5	1	1	1	1	1		
Ewingella americana	10	4	4		1	1			
Hafnia alvei	10	6			3		1		
Klebsiella ornithinolytica	10	6	3	1					
Klebsiella oxytoca	10	5	1	1		3			
Klebsiella pneumoniae subsp. ozaenae	10	3	3		3		1		
Klebsiella pneumoniae subsp. pneumoniae	10	3	4		1	2			
Klebsiella pneumoniae subsp. rhinoscleromatis	10	4	5			1			
Kluyvera ascorbata-K. cryocrescens	10	6	1		1	1		1	
Leclercia adecarboxylata	10	4	4	1	1				
Moellerella wisconsensis	8	7	1						
Morganella morganii	10	6	3			1			
Pantoea agglomerans	7	1	1	3	1			1	
Pantoea dispersa	1	1							
Proteus mirabilis	10	8				2 1			
Proteus penneri	10	2	6			1	1		
Proteus vulgaris	10	8	2						
Providencia alcalifaciens	7	7							
Providencia rettgeri	8		3	1	4				
Providencia rustigianii	2	2							
Providencia stuartii	14	4	8	1	1				
Rahnella aquatilis	2	_	2						
Salmonella enterica subsp. arizonae	10	2	7					1	
Salmonella group	14	11	3					_	
Salmonella group, lactose positive	3							3	
Salmonella serotype Paratyphi A	2	1	_	_		1	_		
Serratia fonticola	10	2	3	2			3		
Serratia liquefaciens group	10	6	2		4	1		1	
Serratia marcescens	10	2	5	2	1				
Serratia odorifera	10	5	3	4	2	2			
Serratia plymuthica	10	3	2	1	1	3			
Serratia rubidaea	10	5	3		1	1	4	4	
Shigella group (three species)	6	3	1		2		1	1	
Shigella sonnei	4	1	4	1	2		1		
Yersinia enterocolitica group (four species)	18	11	4	1	2	1	1	1	
Yersinia pseudotuberculosis	6	1	2			1	1	1	
Total	482	212 (44.0)	126 (26.1)	29 (6.0)	47 (9.8)	34 (7.1)	19 (3.9)	15 (3.1	

^a A total of 85.9% of strains were correctly identified at probability levels ranging from excellent to good.

fermenting and -nonfermenting gram-negative bacilli were taken from the CDC stock culture collection. Ten recently isolated <code>Salmonella</code> strains from hospitals in the Atlanta, Ga., area were obtained from the Georgia Central Public Health Laboratory and passaged a third time on 5% sheep blood agar before use. All incubations were performed at 35 \pm 1°C, unless indicated otherwise. ID-GNB cards were inoculated according to the directions of the manufacturer and

processed in the Vitek 2 instrument with software version VT2-R02.02 (8 June 2000). Final test results were available in approximately $3\ h.$

Media and biochemical tests. Biochemical tests for the identification of enteric organisms were performed with conventional media and by the methods described by Edwards and Ewing (1), with some modifications by Hickman and Farmer (4) and Farmer et al. (2). Biochemical tests for identification of nonen-

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TABLE 2. Enteric strains that were misidentified

17 March 2. Enterio strains that were inisidentalized							
Reference identification	ID-GNB identification (level of accuracy)						
Citrobacter amalonaticus							
Salmonella arizonae, lactose-positive Serratia liquefaciens Shigella flexneri Yersinia pseudotuberculosis	Escherichia coli (very good)Serratia odorifera (excellent)Escherichia coli (good)						

^a LAO, lysine, arginine, and ornithine.

teric organisms were performed by the methods of Weyant et al. (10) and Schreckenberger (9). Commercial media were used whenever possible.

Vitek 2 system. The Vitek 2 system is a fully automated, continuous-access testing system that can accommodate 60 identification or susceptibility cards at one time in one module. Additional modules that extend the capacity in increments of 60 cards are available.

The inoculation of cards begins with the preparation of a standardized bacterial suspension in 0.45% saline equivalent to a McFarland 0.5 to 0.63 standard, the range indicated by the manufacturer. The suspensions are standardized in a

Densi-Chek 2, which is first adjusted with a semisolid calibrator. The suspensions are then placed into the Smart Carrier boat on the Smart Carrier cassette. The boat and cassette each contain a memory chip with information for the suspensions in that load. If a susceptibility test is to be performed simultaneously with the identification, an additional blank tube with 0.45% saline is placed in alternating slots. The time between suspension preparation and card filling for 10 isolates is less than 20 min. Suspensions are prepared in groups of 12 or less.

The ID-GNB cards each have a bar-coded label that is scanned into the memory chip before the cards are loaded into the corresponding slot.

TABLE 3. Nonenteric strains tested with the ID-GNB card

Reference identification	No. (%) of strains identified at the following probability level ^a :									
	n	Excellent	Very good	Acceptable	Good	Low	Unidentified	Error		
Acinetobacter baumanii	6	6								
Actinobacillus ureae	2	2								
Aeromonas hydrophilia-A. caviae	6	6								
Aeromonas sobria	4	2				1		1^b		
Agrobacterium spp.	2			1			1			
Bergeyella zoohelcum	3	3								
Brevundimonas diminuta	2	1				1				
Brevundimonas vesicularis	2	1						1^c		
Burkholderia cepacia	7		3	1	1	2				
Chromobacterium violaceum	4	3				1				
Chryseobacterium indologenes	4		2	1	1					
Chryseobacterium meningosepticum	3	3								
Empedobacter brevis	2	1						1^d		
Myroides spp.	2					2				
Ochrobactrum anthropi	2 2 3 2			1		1				
Pasteurella aerogenes	3	2	1							
Pasteurella haemolytica	2		1		1					
Pasteurella multocida	3	3	-		-					
Pasteurella pneumotropica	2	1		1						
Plesiomonas shigelloides	5	4	1	-						
Pseudomonas aeruginosa	10	9	1							
Pseudomonas luteola	2	1	-		1					
Pseudomonas oryzihabitans	2	1	1		1					
Ralstonia pickettii	5	1	2		1		1			
Shewanella putrefaciens	3	2	1		1		1			
Sphingobacterium multivorum	2	1			1					
Sphingomonas paucimobilis	2	1		1	1					
Stenotrophomonas maltophilia	8	5		1	1	2				
Weeksella virosa	3	3			1	2				
Total	103	61 (59.2)	13 (12.6)	6 (5.8)	8 (7.8)	10 (9.7)	2 (1.9)	3 (2.9)		

^a A total of 85.4% of strains were correctly identified at probability levels ranging from excellent to good.

^b Identified as Aeromonas hydrophila-A. caviae (excellent).

^c Identified as *Chryseobacterium indologenes* (low).

^d Identified as *Chryseobacterium indologenes* (very good).

Authors (reference)	No. of	Percent							
	strains tested	Correct	Correct with additional tests	Indeterminate	No identification	Error			
Funke et al. (3)	845	84.7	3.8	9.5	1.2	0.8			
Jossart and Courcol (5)	502	85.7		11.0	1.2	2.2			
O'Hara and Miller (this study)	585	85.8		7.5	3.6	3.1			
Joyanes et al. (6)	198^{a}	66.6		24.2	8.6	0.5			
Ling et al. (7)	281	95.0			2.8	2.1			
Sanders et al. (8)	211	93.3	0.9		2.4	3.3			

TABLE 4. Other published evaluations of the ID-GNB card and Vitek 2 instrument

Once the Smart Carrier boat is loaded into the Vitek 2 instrument, the instrument automatically reads the information from the memory chip, makes the dilution that is necessary if a susceptibility test is to be included, fills the test cards, severs the filling straws from the cards, and incubates the cards for 3 h. When the process is complete, onboard software and automation move the cards to the discard area, analyze the data, and print the results.

ID-GNB card. The ID-GNB card is a 64-well card that contains 41 biochemical tests (13 more substrates than the number included on the GNI+ card) and two negative control wells. Of the 41 biochemical tests, 21 are with conventional substrates that include adonitol, L-arabinose, D-cellobiose, D-galacturonate, D-glucose, D-glucuronate, *myo*-inositol, D-maltose, D-mannitol, D-melibiose, palatinose, D-raffinose, L-rhamnose, saccharose, D-sorbitol, D-trehalose, lysine and ornithine decarboxylases, urease, malonate, and tryptophane deaminase. Twenty wells contain preformed enzymes. These include glucose-1-phosphate, 5-keto-D-gluconate, α-arabinosidase, α-galactosidase, α-glutamate, β-cellobiosidase, β-galactosidase, β-glucoridase, β-β-glucoridase, β-β-glucoridase, β-N-acetyl-glucosaminidase, β-N-acetyl-glacosaminidase, β-N-glutamyl-transferase, L-lysine arylamidase, phosphatase, L-prolidonyl arylamidase, and CBZ-arginine arylamidase. The remaining wells are empty. Differences in the substrates between this card and the GNI+ card can be seen by consulting the respective product inserts.

Occasionally, additional off-line same-day testing may be required to complete an identification. Those tests listed by the manufacturer for the completion of an identification were performed with CDC conventional biochemicals. These included tests for indole production, motility, oxidase, β -hemolysis, and determination of brown, orange, purple, red, or yellow pigments.

Taxonomy. Organism identifications were taken from the Vitek database, even though some taxonomic changes may have occurred since the database was compiled.

Classification of answers. Answers may be categorized into one of several confidence levels (excellent, very good, good, acceptable), all of which are an estimate of how closely a given profile corresponds to a particular taxon relative to all the other taxa in the database. The percentage of identification ranges from 99.9 to 80.0%. This value is extrapolated from the *t* index, which is an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon and which is actually calculated from the algorithm. The value of the *t* index varies between 0 and 1 and is inversely proportional to the number of atypical tests. Thus, a confidence level of excellent is a combination of a percentage of identification of ≥99.9% and a *t* index of ≥0.75. A confidence level of acceptable combines a percentage of identification of ≥80.0% and a *t* index of ≥0. In the case of a low level of discrimination, supplementary tests are proposed.

RESULTS AND DISCUSSION

The 482 enteric strains tested in this study included 21 genera and 60 species (Table 1). They represented most major species of the family *Enterobacteriaceae*, and all were of human origin. Because the CDC is usually not given patient histories when isolates are submitted, it is not known if they were clinically relevant, but most of the sources would indicate that the possibility of clinical relevance might exist. At the end of the initial 3-h incubation period, 414 (85.9%) isolates were correctly identified at confidence levels ranging from excellent to

good. Another 34 (7.1%) were correctly identified, but at a low level of discrimination. In the clinical setting, these results would have to be carefully evaluated to determine if they were correct. The Vitek 2 instrument could not identify 19 (3.9%) of the 482 isolates and gave an answer of "unidentified organism." Fifteen (3.1%) strains were incorrectly identified (Table 2), although six were at least in the correct genus. Most of these incorrect identifications were at the low level of discrimination.

Of special interest in Table 2 are the lactose-positive *Salmonella* strains, one of which was *Salmonella enterica* subsp. *arizonae*. All four of these isolates were identified as *Escherichia coli* at the very good probability level.

Table 3 shows the test results for 103 isolates of nonenteric gram-negative bacilli that were a mixture of glucose-fermenting and glucose-nonfermenting, oxidase-positive and oxidase-negative organisms. They include 21 genera and 30 species. At the end of the initial incubation period, 88 (85.4%) strains were correctly identified at probability levels ranging from excellent to good. Ten (9.7%) were correctly identified, but at a low level of probability, and three (2.9%) were misidentified. The latter three included one isolate each of *Aeromonas sobria*, *Brevundimonas vesicularis*, and *Empedobacter brevis*. Two (1.9%) strains were not identified.

Table 4 presents a comparison of the results of this study and those of the four other studies of the Vitek 2 instrument that have been published. There is no significant difference in the results obtained in this study compared with those obtained by Funke et al. (3) or Jossart and Courcol (5), although the smaller studies of Joyanes et al. (6), Ling et al. (7), and Sanders et al. (8) showed significantly better results.

Table 5 shows a comparison of the results obtained when 454 of the same strains listed in Table 1 were tested with the Vitek GNI+ card. Table 5 lists those strains that were correctly identified, unidentified, or identified in error. The 25 strains in the tests with the ID-GNB card and the 8 strains in the tests with the GNI+ card for which there was a low probability of correct identification are not listed in Table 5. Even though the error rate was higher (4.6%) with the GNI+ card, only 1.7% of the identifications had low-probability confidence levels, whereas with the ID-GNB card, 5.5% of the identifications had low-probability confidence levels. Thus, there is no significant difference in the abilities of these two cards to identify these strains accurately (Yates' corrected *P* value, >0.05).

The question of the relative acceptable level of accuracy when a "system" approach is used for identification is often

^a The study included only Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia.

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TABLE 5. Comparison of levels of accuracy of identification between ID-GNB and GNI+ cards^a

Reference identification		No. o	of isolates with the	indicated id	dentification with the following card ^b :			
	Total no. of isolates tested		ID-GNB	GNI+				
		Correctly identified	Unidentified	Error	Correctly identified	Unidentified	Erro	
Cedecea davisae	6	5	1		6			
Cedecea lapagei	4	4			3	1		
Citrobacter amalonaticus	10	7		1	10			
Citrobacter braakii	5	5			3		1	
Citrobacter farmeri	5	3			4	1		
Citrobacter koseri	10	9	1		9	1	_	
Citrobacter youngae	4	3			2		2	
Edwardsiella tarda	10	7		1	8	1	1	
Enterobacter aerogenes	10	9	1		10	_		
Enterobacter asburiae	10	9	1		8	2		
Enterobacter cancerogenus	10	7	1	1	10			
Enterobacter cloacae	10	6		1	9			
Enterobacter gergoviae	10	10			9		1	
Enterobacter sakazakii	10	10	-		10		-	
Escherichia coli	30	24	3	1	27		2	
Escherichia fergusonii	10	10			8	1		
Escherichia hermannii	10	9	1		9	_	1	
Escherichia vulneris	10	8	1		7	2	1	
Ewingella americana	10	9			10			
Hafnia alvei	9	9			7	1	1	
Klebsiella ornithinolytica	10	10			10			
Klebsiella oxytoca	10	7			10			
Klebsiella pneumoniae subsp. ozaenae	10	9	1		10			
Klebsiella pneumoniae subsp. pneumoniae	10	8			10			
Klebsiella pneumoniae subsp. rhinoscleromatis	10	9			10		_	
Kluyvera ascorbata-K. cryocrescens	10	8		1	2	1	2	
Leclercia adecarboxylata	8	8			7		1	
Moellerella wisconsensis	8	8			7	1		
Morganella morganii	10	9			10	_		
Pantoea agglomerans	7	6		1	5	2		
Proteus mirabilis	10	8			10			
Proteus penneri	8	6	1		7	1		
Proteus vulgaris	9	9			9			
Providencia alcalifaciens	7	7			6	1		
Providencia rettgeri	8	8			7	1		
Providencia rustigianii	2	2			2			
Providencia stuartii	14	14			14			
Rahnella aquatilis	2	2			2		_	
Salmonella enterica subsp. arizonae	7	6		1	5		2	
Salmonella group	0	0			0			
Salmonella enterica serotype Cholerae-suis	2	2			1	1		
Salmonella serotype Paratyphi A	2	1			2			
Salmonella enterica serotype Typhi	2	2			2			
Salmonella enterica serotype Typhimurium	1	1			1			
Serratia fonticola	10	7	3		8	1	2	
Serratia liquefaciens group	10	8		1	9		1	
Serratia marcescens	10	10			9		1	
Serratia odorifera	10	10			10			
Serratia plymuthica	10	7			9	1		
Serratia rubidaea	10	9			10			
Shigella group (three species)	6	4	1	1	5		1	
Shigella sonnei	4	3	1		4			
Yersinia enterocolitica group (four species)	18	18			16	1	1	
Yersinia pseudotuberculosis	6	3	1	1	5	1		
Total	454	392	18	11	403	22	21	

^a Numbers in the correctly identified, unidentified, and error columns may not add to the total number. Identifications at the low level of accuracy were not included. ^b A total of 410 (90.3%) strains were correctly identified with the ID-GNB card (error rate, 2.4%), whereas a total of 425 (93.6%) strains were correctly identified with the GNI card (error rate, 4.6%).

posed. This is a decision that must be made by each laboratory after it takes into consideration many variables. The two most prominent of these variables are the source of the specimen and whether the susceptibility pattern matches the identifica-

tion of the organism. For example, an identification of *Pantoea agglomerans* from urine might not merit the same amount of concern that it would if it was isolated from blood or spinal fluid. Some laboratories are not willing to accept an answer

that has less than an 85% probability of being accurate; others want an answer that is at least 90% accurate. Another consideration is whether the laboratory is able or willing in terms of time and money to perform the additional testing needed.

The hands-off approach to the Vitek 2 instrument with the Smart Carrier station is a major improvement of the Vitek instrument. Having demonstrated an overall accuracy above 90%, testing with the ID-GNB card presents an acceptable method for the identification of the most commonly isolated members of the family *Enterobacteriaceae* and nonenteric gram-negative bacilli.

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